

PATENT COOPERATION TREATY

1986(11)

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day month year) 14 August 2000 (14.08.00)	
International application No. PCT/IB99 02053	Applicant's or agent's file reference F7.204/PCT
International filing date (day month year) 24 December 1999 (24.12.99)	Priority date (day month year) 24 December 1998 (24.12.98)
Applicant FELL, Claude	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
05 July 2000 (05.07.00)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41) 221 746 14 39

Authorized officer

Zakaria EL KHODARY

Telephone No. (41) 221 3566 6 56



PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference F7.204/PCT	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IB99/02053	International filing date (day/month/year) 24/12/1999	Priority date (day/month/year) 24/12/1998	
International Patent Classification (IPC) or national classification and IPC A61M1/36			
Applicant BIOSAFE S.A. et al.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 6 sheets, including this cover sheet.

- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 05/07/2000	Date of completion of this report 30.01.2001
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bichlmayer, K-P Telephone No. +49 89 2399 2977



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IB99/02053

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

Description, pages:

1-3,5-12,14-18	as originally filed		
4,13	as received on	08/01/2001	with letter of 08/01/2001

Claims, No.:

1-22	as received on	08/01/2001	with letter of 08/01/2001
------	----------------	------------	---------------------------

Drawings, sheets:

1/7,5/7-7/7	as originally filed		
2/7-4/7	as received on	08/01/2001	with letter of 08/01/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IB99/02053

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-22
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IB99/02053

Ad section V:

- 1) Independent claim 1

Reference is made to the following document/s/:

D1: WO,A,9 715 399

D2: US,A,3 737 096

D1 discloses a cell separation system for biological fluids which is used in a combined mode of a transfer step for introducing the mixture to be separated into a separation chamber, a step for separating the components by centrifugation and a step for extracting the separated components from said separation chamber into containers. The system of D1 comprises (cf. Figs. 1 and 3, respectively) a centrifugal processing chamber (4), a set of containers (83-85), an axially movable member (5), a separation space (12), distribution valve arrangement (87-89), means (50) for monitoring the position of the movable member and means (control system 74) for controlling the system in the combined mode cited above. Nowhere in D1 a separate transfer mode as defined in independent claim 1 is disclosed or suggested.

Thus, the subject-matter of the independent claim 1 is distinguished over D1 in that said means for controlling of the system are adapted to perform its function also with respect of said transfer mode, i.e. to intake and express fluid with the separation chamber stationary, the valve actuation, the axial movement of the member (21) and the monitoring of the position of said member. Therefore, claim 1 meets the requirements of Art. 33(2) PCT.

As effect of the distinguishing features usability of the system known from D1 is enlarged in that for example fluid transfer from one of said containers to others can be realized simply by movement of said moveable member (21) and acting the separation chamber as intermediate container. The objective problem to be solved by the present application can therefore be considered as providing an apparatus for processing and separation of biological fluids into components and including general laboratory processing.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IB99/02053

None of the available prior art documents discloses or suggests the distinguishing features to solve the objective problem. D1 mentions at page 11, that an anticoagulant solution can be introduced into said separation chamber by moving the moveable member downwards but the components containing this fluid are all transferred from the chamber into said containers during rotation of the separation chamber. According to D2 concerning a blood processing control apparatus, wash solutions are transferred from separate containers into a separation centrifuge and transferred back into a waste container. However, although said centrifuge is stopped before said transfer is initiated, said wash fluid flows passively from the respective container into said separation centrifuge without any movement of the flexible membrane (7) so that D2 also does not make the solution provided by claim 1 obvious.

Thus, independent claim 1 meets the requirements of Art. 33(3) and (4) PCT.

2) Dependent claims 2 to 12

The dependent claims 2 to 12 concerns specific embodiments of the inventive concept of independent claim 1 and meet therefore also the requirements of Art. 33(2) to (4) PCT.

3) Independent claim 13

The method claimed in claim 13 concerns a method of processing and separating biological fluids wherein the system of the claims 1 to 12 is used in this method. Therefore, claim 13 also meets the requirements of Art. 33(2) to (4) PCT for the same reasons set out under point 1) above.

4) Dependent claims 14 to 16

Claims 14 to 16 depend directly or indirectly from the independent claim 13 and meet as such also the requirements set out in Art. 33(2) to (4) PCT.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IB99/02053

5) Independent claim 17

The set claimed in independent claim 17 contains the novel and inventive subject-matter of the novel and inventive claims 1 to 12 and therefore meets also the requirements of Art. 33(2) to (4) PCT.

6) Dependent claims 18 to 19

Dependent claims 18 and 19 are directed to specific embodiments of the independent claim 17 and meet as such also the requirements of Art. 33(2) to (4) PCT.

7) Independent claim 20

Use of the system claimed in claims 1 to 12 as defined in independent claim 20 fulfills also the requirements of Art. 33(2) to (4) PCT insofar as the system of claims 1 to 12 is novel and inventive as set out under point 1) above.

8) Dependent claims 21 to 22

Dependent claims 21 to 22 are directed to specific embodiments of the independent claim 20 and meet as such also Art. 33(2) to (4) PCT.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference F7.204/PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/IB 99/02053	International filing date (day/month/year) 24/12/1999	(Earliest) Priority Date (day/month/year) 24/12/1998
Applicant BIOSAFE S.A. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

3

☐ None of the figures.



INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/IB 99/02053

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61M1/36 B04B5/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61M B04B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 15399 A (FELL CLAUDE) 1 May 1997 (1997-05-01) page 13, line 3 - line 10; claims	1,5, 8-10,14 12 13
Y	US 5 368 542 A (MCMANNIS JOHN D ET AL) 29 November 1994 (1994-11-29) claims 1-8	12
A	US 5 641 622 A (LAKE WILLIAM C ET AL) 24 June 1997 (1997-06-24)	15,16
A	US 3 737 096 A (JONES A ET AL) 5 June 1973 (1973-06-05) abstract	1

	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"S" document member of the same patent family

Date of the actual completion of the international search

28 March 2000

Date of mailing of the international search report

05/04/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer

Villeneuve, J-M

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/IB 99/02053

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>US 4 946 434 A (LUEPTOW RICHARD M ET AL) 7 August 1990 (1990-08-07) abstract; figures</p>	2,3

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Application No

PCT/18 99/02053

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9715399	A	01-05-1997	AT 186235 T	15-11-1999
			AU 6467796 A	15-05-1997
			DE 69605062 D	09-12-1999
			EP 0912250 A	06-05-1999
US 5368542	A	29-11-1994	US 5316540 A	31-05-1994
US 5641622	A	24-06-1997	US 5916743 A	29-06-1999
			CA 2140455 A	08-12-1994
			EP 0653062 A	17-05-1995
			JP 9506501 T	30-06-1997
			WO 9427698 A	08-12-1994
US 3737096	A	05-06-1973	CA 990255 A	01-06-1976
			DE 2254403 A	12-07-1973
			FR 2164621 A	03-08-1973
			GB 1371678 A	23-10-1974
			IT 971134 B	30-04-1974
			JP 1100988 C	18-06-1982
			JP 48071093 A	26-09-1973
			JP 56036943 B	27-08-1981
US 4946434	A	07-08-1990	WO 8901792 A	09-03-1989
			AT 64863 T	15-07-1991
			EP 0257755 A	02-03-1988
			JP 1851493 C	21-06-1994
			JP 5058346 B	26-08-1993
			JP 63065873 A	24-03-1988
			US 4943273 A	24-07-1990
			US 4983158 A	08-01-1991



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



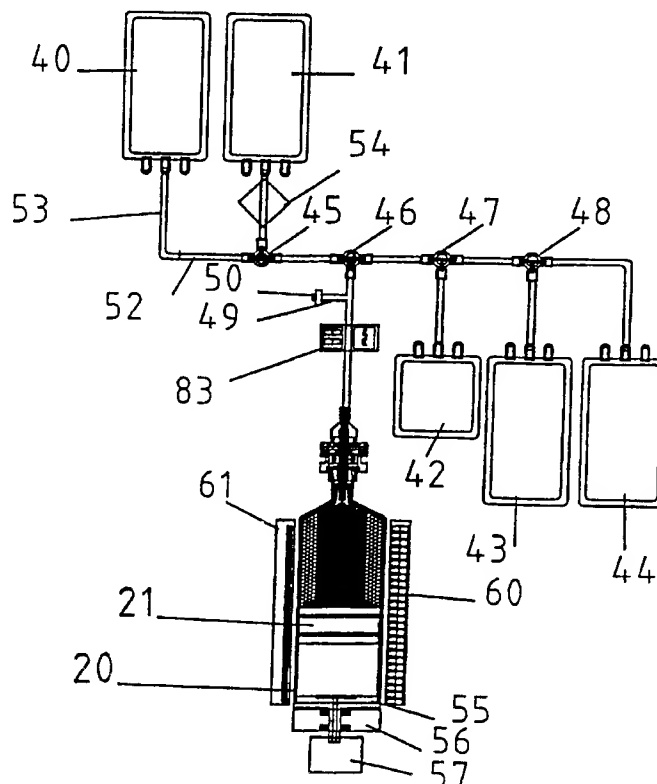
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61M 1/36, B04B 5/04		A1	(11) International Publication Number: WO 00/38762
			(43) International Publication Date: 6 July 2000 (06.07.00)
(21) International Application Number: PCT/IB99/02053		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 24 December 1999 (24.12.99)			
(30) Priority Data: PCT/IB98/02114 24 December 1998 (24.12.98) IB			
(71) Applicant (for all designated States except US): BIOSAFE S.A. [CH/CH]; Rue de l'Aiselet, CH-1262 Eysins (CH).			
(72) Inventor; and (75) Inventor/Applicant (for US only): FELL, Claude [CH/CH]; 22, Route du Stand, CH-1260 Nyon (CH).			
(74) Agents: CRONIN, Brian et al.; Moinas Savoye & Cronin, 42, rue Plantamour, CH-1201 Geneva (CH).			
		Published With international search report.	

(54) Title: BLOOD SEPARATION SYSTEM PARTICULARLY FOR CONCENTRATING HEMATOPOIETIC STEM CELLS

(57) Abstract

A system for separating biological fluids into components, comprises a set of containers (40, 42-44) for the biological fluid to be separated and the separated components, optionally an additional container (41) for additive solution, and a hollow centrifugal processing chamber (20) having an axial inlet/outlet (7) for the biological fluid. The processing chamber contains a piston (21) movable to intake a selected quantity of biological fluid and express processed biological fluid components via the outlet. Optical means (60-69) monitor the position of piston (21) to control the amount of intaken fluid and the expression of components. A distribution valve arrangement (45-48) selectively communicates the processing chamber (20) and the containers (40-44) or places them out of communication. The system is arranged to operate in a separation mode and in a non-separation transfer mode, especially for adding preservative solution to separated blood stem cells. In the separation mode, fluids are intaken into processing chamber (20), centrifuged and separated into components, and the components expressed possibly through the use of a density gradient product. In the transfer mode, the processing chamber (20) intakes and expresses fluid with the chamber stationary, the valve actuation arrangement (45-48) transferring fluid from one container (40-44) to another via the processing chamber (20), by movement of the piston (21), without centrifugation or separation, and the means (60-69) for monitoring the piston's position controls the amounts of non-separated fluids transferred.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

Blood Separation System Particularly for Concentrating
Hematopoietic Stem Cells

FIELD OF THE INVENTION

5 This invention relates to the automated processing
and separation of biological cells as found in whole
blood, and relates more specifically to a functionally
closed system allowing to extract certain cell populations
like hematopoietic stem cells, for immediate use or their
10 mixing with an additive solution or a storage solution for
later separate storage operations and to the methods for
carrying out such an extraction.

BACKGROUND OF THE INVENTION

15 Blood separation systems and methods have emerged
over the past 20 years in response to the growing need for
efficient blood component therapies. Among them are the
transplantation of hematopoietic progenitor stem cells,
which in many cases is the only remaining cure to
oncological disorders. Patients in need of a stem cell
20 transplant have mainly three options:

- 1) Adult bone marrow stem cells;
- 2) Peripheral blood stem cells found in the
circulatory system;
- 3) Stem cells found in umbilical cord and placental
25 blood retrieved at birth of a new born infant.

For most stem cell transplants, the main limitation
has been the risk of graft-versus-host-disease (GVHD),
requiring an excellent HLA-match tissue (HLA = Human
Leucyte Associated).

30 Umbilical cord blood is a rich source of the
primitive hematopoietic stem and progenitor cells, with
extensive proliferation capacity and capacity to self-

renew. This field has advanced rapidly from clinical implants utilizing only HLA-matched grafts to unrelated donor cell transplants which open a much larger indication for stem cell transplantation. This increase in clinical
5 experience with cord blood is due mainly to the establishment of banks for storage of hematopoietic stem cells from unrelated umbilical blood cord.

Blood volumes recovered from umbilical cord are usually very low (40 to 150 ml) and there is some concern
10 that any attempt at product manipulation and concentration might result in stem cell loss, which might impair engraftments. Therefore umbilical cord blood is sometimes stored as is, with preservative solution added. A much preferred way would be to eliminate most unwanted cells
15 like red cells and white cells, resulting in a considerable volume reduction. Less preservative solution would be required, smaller bags, smaller storage spaces would be used and considerable energy savings achieved, all of this translating in substantial cost savings. The
20 quality of the stem cell product when retransfused would be improved as well, as lysed cells resulting from storage would be drastically reduced.

No device or automated system exists for processing and concentrating on line umbilical cord stem cells. There
25 is nevertheless a considerable interest for concentrating umbilical cord blood stem cells without loss or altering their functionality.

EP-B-0 912 250 (C.FELL), the contents whereof are herein incorporated by way of reference, describes a
30 system for the processing and separation of biological fluids into components, comprising a set of containers for receiving the biological fluid to be separated and the separated components, and optionally one or more additional containers for additive solutions. A hollow

centrifuge processing chamber is rotatable about an axis of rotation by engagement of the processing chamber with a rotary drive unit. The processing chamber has an axial inlet/outlet for biological fluid to be processed and for processed components of the biological fluid. This inlet/outlet leads into a separation space of variable volume wherein the entire centrifugal processing of biological fluid takes place. The processing chamber comprises a generally cylindrical wall extending from an end wall of the processing chamber, this generally cylindrical wall defining therein the hollow processing chamber which occupies a hollow open cylindrical space coaxial with the axis of rotation, the axial inlet/outlet being provided in said end wall coaxial with the generally cylindrical wall to open into the hollow processing chamber. The processing chamber contains within the generally cylindrical wall an axially movable member such as a piston. The separation space of variable volume is defined in an upper part of the processing chamber by the generally cylindrical wall and by the axially movable member contained in the generally cylindrical wall of the processing chamber, wherein axial movement of the movable member varies the volume of the separation space, the movable member being axially movable within the processing chamber to intake a selected quantity of biological fluid to be processed into the separation space via the inlet before or during centrifugal processing and to express processed biological fluid components from the separation space via the outlet during or after centrifugal processing. Means are provided for monitoring the position of the movable member to thereby control the amount of intaken biological fluid and the expression of separated components. The system further comprises a distribution valve arrangement for establishing selective communication between the processing chamber and selected containers or

for placing the processing chamber and containers out of communication.

The system according to EP-B-0 912 250 is designed to operate for the separation of biological fluids, and has
5 proven to be very polyvalent for many separation applications, especially for on-line separation of components from a donor or a patient.

DISCLOSURE OF THE INVENTION

According to the invention, such system is arranged
10 to operate in a separation mode as defined in claim 1 and in a non-separation transfer mode, which provides greater possibilities for use of the system including new applications which were heretofore not contemplated, such as separation of hematopoietic stem cells and in general
15 laboratory processing. According to the invention, the system is arranged to operate such that:

- in the separation mode fluids can be intaken into the processing chamber while the chamber is rotating or stationary, fluid intaken into the chamber is centrifuged
20 and separated into components, and the separated components expressed while the chamber is rotating or, optionally, for the last separated component, while the chamber is stationary; and

- in the transfer mode the processing chamber intakes
25 fluid and expresses fluid with the chamber stationary. The valve actuation arrangement is actuatable to transfer amounts of fluid from one container to another via the processing chamber, by moving the member, without centrifugation or separation of the fluid into components,
30 and the means for monitoring the position of the movable member controls the amounts of non-separated fluids transferred.

Further features of the invention are set out in the claims. This invention thus proposes a functionally closed processing kit associated with a portable apparatus, whose function is to monitor and automate the procedure. The kit, usually disposable for avoiding the likelihood of disease transmission, is based on a centrifugal processing chamber whose volume can be varied during operation, allowing to adjust to the exact quantity of blood to process. Such variable volume chamber is described in the aforementioned EP-B-0 912 250 (C.FELL). The chamber is connected to a set of bags and tubing lines for the collection of the separated components. The blood bag containing the blood to process is generally connected to the disposable set through the use of a sterile connecting device, or an aseptic connection under laminar flow. It is however possible to have this bag prefilled with anticoagulant and preconnected to the disposable kit.

A bag containing an additive solution can be connected to the disposable kit via a bacterial filter. The other bags are provided for the collection of the separated components. The stem cell collection bag material is optimally chosen for the storage conditions.

The tubing line selection for conveying the separated products into the proper bags is accomplished by a set of rotational valves called stopcocks that can be arranged in a manifold array, or by a single multiport rotational valve, forming part of the set. Such an arrangement allows to eliminate any cross-contamination between adjacent lines when using standard pinch valves.

The above-mentioned disposable kit cooperates with an instrumentation for monitoring and automating the process, for instance as described in EP-B-0 912 250 (C.FELL). The centrifuge drives a rotating disk which receives the centrifugal processing chamber and locks it in place. Its

closing cover will grip and hold the housing of the rotary seal of the processing chamber.

An optical sensor made of an array of LED and corresponding receiving sensors placed at 180° is implemented vertically on the side of centrifuge, for monitoring the piston position. Volumes intaken into or extracted from the chamber can therefore be exactly measured. The topdeck receives an optical line sensor module which monitors the color in the effluent tubing, feeding-back this information to the control program. An array of shafts equipped with fittings for driving a set of multiple stopcock valves protrude from the top deck. They are coupled to a set of motors enabling the tubing line selection. Encoders are attached to the motors for monitoring the stopcock valves position. The front panel incorporates a window allowing the user to see displacement of the piston in the chamber.

The procedure for extracting stem cells out of an umbilical cord is as follows. Initially blood is recovered from the umbilical cord at birth and collected aseptically into a plastic bag, with anticoagulant added like Citrate-Phosphate-Dextrose CPD-1 to avoid clotting. After initial sampling is taken to assess its richness in stem cells, the bag is sterile or aseptically connected to the processing kit and the whole set is loaded onto the separation system, which initially operates in the separation mode or the transfer mode at choice. In the separation mode the centrifuge starts driving the separation chamber at around 4000 rpm, and blood is introduced by moving down the chamber piston pneumatically. Two cases can then occur. If the volume of blood to process is smaller than the processing chamber volume (as detected by the empty state of the effluent tubing), the piston is maintained at an intermediate position pneumatically, monitored by the piston position

sensor. If the volume of blood completely fills the separation chamber, detected by the piston reaching the bottom of the chamber, the pneumatic compressor stops. In both cases, centrifugation speed is increased to around
5 6000 rpm to shorten the sedimentation time to 5-8 min. After this period, centrifugation slowly decreases to around 4000 rpm. Stopcocks are rotated to allow the collection of the separated products, and the pneumatic pressure gradually increases to move the piston upwards.
10 The speed of the piston remains low, actively monitored by the piston position sensor, in order to maintain the sedimentation profile of the cells within the chamber. The first milliliters from the inlet line are purged in the stem cell bags. Plasma is extracted then collected into a
15 first bag. It is followed by platelets, packed in the intermediate layers, or buffy-coat. Apparition of the first platelets is detected by the optical line sensor monitoring the effluent line tubing. At that moment, the product, very rich in stem cells, is directed into a
20 second collection bag by rotating a stopcock valve. A volume counter is started, which depends among other factors on the total blood volume processed. When this counting volume has been reached, centrifugation is stopped. The proper stopcock valve is rotated and the last
25 product is extracted, essentially a volume of packed red cells with residual granulocytes, into a third collection bag. Another cycle can then resume if the umbilical cord blood has not been totally processed. Otherwise, the separation and stem cells collection process is completed
30 at this stage.

However, it is possible to reprocess the content of the bag containing the stem cells, in view of further purifying the product. In this case, the proper stopcock valve is rotated to intake the content of the stem cell
35 concentrate bag. The procedure to collect stem cell rich layer is identical then to the one described above.

Another alternative to isolate the stem cell rich fraction from the buffy-coat is by using density gradient products such as those available under the names Ficoll and Percoll. In this alternative, a density gradient product is first introduced into the processing chamber, followed by introduction of whole blood, and a component of the biological fluid is separated into a given container and its collection is completed when the density gradient appears. Possibly the density gradient product may be introduced during processing.

Using Ficoll would for example consist of first introducing the density gradient into the processing chamber, followed then by whole blood. After complete introduction of blood into the chamber, a sedimentation period of a few minutes is started. Stem cells and platelets form an interface in front of the gradient, whereas erythrocytes and granulocytes have passed through the Ficoll and are held against the walls of the separation chamber. The piston is then lifted gently as in the standard procedure, the stem cell fraction being collected at the apparition of the first platelets. The effluent line clears up again when Ficoll exits the chamber, which is the appropriate moment to stop the collection.

When the stem cells are collected by one of the methods described above adequate preservative solution can be introduced into the processing chamber by rotating the proper stopcock, the system operating in the transfer mode. It is then retransferred into the stem cell bag, its volume accurately controlled by the piston position sensor.

The bag containing the stem cell rich product can be disconnected at this stage from the rest of the set. Its volume ranges between 20-40 ml, depending of the initial

volume processed. The by-products of the separation, plasma and packed red cells, can then be used for serology and HLA typing, avoiding any product loss due to sampling in the stem cell bag.

5 This separation system and method offer significant advantages over manual processing techniques. The disposable kit is a functionally closed system, avoiding any risk of contaminating the product during manipulation. The protocol is fully automated, through a microprocessor
10 based control system, with ability to vary the main parameters, like centrifugation speed, centrifugation time, speed of introduction and extraction, volume to collect, etc. The volume reduction for the stem cell product represents a gain of 50% at least compared with
15 the current state of the art. The instrumentation is very compact and portable, ideal for the decentralized processing of such procedures.

 A further aspect of the invention is the use of the above-described system for processing variable volumes of
20 biological fluid from 10 ml up to the maximum volume of the separation chamber, and for adding an additive solution to the separated components, in particular for separation of stem cells from blood and mixing the separated stem cells with a preservative solution; for
25 separation of hematopoietic stem cells from umbilical cord blood; for separation of hematopoietic stem cells from an apheresis collection; and for separation of hematopoietic stem cells from a bone marrow aspirate.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be further described by way of example with reference to the accompanying drawings in which:

- 5 - Fig. 1 is a schematic side elevation and cross-sectional view of a processing chamber and its rotary seal, showing the various sedimentation layers of blood components.
- 10 - Fig. 2 is a schematic side elevation and cross-sectional view of the processing chamber and its rotary seal, associated with an optical sensor for monitoring the piston sensor, and control circuitry.
- 15 - Fig. 3 illustrates in a schematic form the disposable set carrying a manifold stopcock system for the processing and separation of umbilical cord blood.
- Fig. 4 illustrates in a schematic form an array of stopcocks arranged in a manifold.
- 20 - Fig. 5 is a schematic side elevation and cross-sectional view of the motor driver and control elements for rotating each individual stopcock.
- Fig. 6 is a schematic side elevation and cross-sectional view of a multiport rotational valve.
- 25 - Fig. 7 schematically illustrates the multiport rotational valve of Figure 6 with an inlet/outlet port located at the center and a range of associated ports located at the periphery.
- Fig. 8 is perspective view of a cabinet containing instrumentation and devices for controlling the processing.

- Fig. 9.1 to Fig. 9.6 are functional diagrams showing the various steps of umbilical cord blood separation using a disposable set including a processing chamber and a set of stopcocks according to the invention.

5

DETAILED DESCRIPTION OF THE SYSTEM

The processing chamber 20 is in accordance with that described in EP-B-0 912 250 (C.FELL). Fig. 1 is a general view of the processing chamber 20. A rotary seal 12 is located on its upper extremity 10. The rotary seal 12 is composed of an upper body 1 and lower body 2. In between is located a friction disk 3, made of a generally low friction material like polished stainless steel or ceramic. A central tubing 7 made of biocompatible material like polycarbonate is attached to the upper body 1. An O-ring 8 ensures airtightness between the upper body 2 and friction disk 3. The rotary seal 12 is mounted on a central bush 11 fitted on the upper extremity 10 of processing chamber 20. However, central bush 11 can be an integral part of chamber 20. The gap between walls of central tubing 7 and central bush 11 is small, say 0.5 mm, to provide a high rotational impedance for stopping any liquid to reach the upper extremity of bush 11. A ball bearing 9 is mounted on bush 11 to ensures the proper alignment of the processing chamber 20 when inserted into centrifuge assembly. Two rubber seals 5,6 are located on either side of the friction disk 3, seal 5 being on the upper side and seal 6 being on the lower side. The seals 5 and 6 are of the V-seal type and ensure airtightness both in positive and negative pressure, up to at least ± 0.5 bar.

The piston 21 is made of a transparent material like polycarbonate and is equipped with two O-rings 24 and 25. These O-rings are made of low friction material like silicon. The processing chamber 20 is closed on its bottom

side by a cap 22 carrying a bacterial filter 23. Air can pass through central opening 26 and filter 23 in cap 22. The position of the piston 21 can be accurately monitored by an optical sensor assembly 60 and 61 (Fig 2). Assembly
5 61 is made of a vertical array of LED, preferably with light emitting in the infrared spectrum to reduce disturbance from ambient light. Only the LED facing piston 21 are turned on, in order to avoid interference from the other LED. The beam of light crosses the transparent
10 piston 21, between the two O-rings 24 and 25. A CCD ("Charge Coupling Device") linear array 61 is placed at 180° on the other side, the exposed pixels of array 61 generating a signal 62 in the form of a peak.

Signal 62 is fed to a low-pass filter 69 and the
15 filtered signal fed to a comparator 67 which also receives from potentiometer 68 a threshold value for discriminating the filtered signal from ambient noise. The output of comparator 67 is connected to the enable gate of counter 65. Clock signal 66 is used to intake the response from
20 each individual pixel of the CCD linear array 61, and feed this to the input of counter 65. The output of counter 65 is connected to a CPU 64 which calculates the position of piston 21 and, when required, shifts the turned-on LEDs 60 via a multiplexer/LED driver 63. Similarly, when
25 necessary, the CPU 64 will vary the signal of compressor driver 70 that supplies compressor 71 in order to increase or decrease pressure applied below the piston 21 to control its position.

This is only one example of position sensing for the
30 piston 21. The light source 60 could be a filament bulb, or a unique linear source of light. The CCD linear array 61 could be replaced by an array of photosensing devices. The receiving sensing device (61) could be placed also beside the emitting light device 62, the system working in

reflection light from the piston 61 rather in transmittance light through the piston 21.

The disposable set (Fig 3) is composed of bags 40-44, tubing lines connected to stopcocks 45-48, and the processing chamber 20. Bag 40 contains the umbilical cord blood to process. Bag 41 contains preservative solution, generally based on a DMSO (Dimethyl Sulfoxide) solution. It is connected to the disposable set through a bacterial 0.2 micron filter 54. Bag 42 is the collection bag for the stem-cell rich product. Its plastic composition is made of a material suiting long term storage. Bag 43 is the collection bag for the plasma and bag 44 is the one for the red cells.

An array of stopcocks 45-48 (Fig. 5) organized in a manifold 58 allows the connection between the different tubing lines. These rotational stopcocks provide an excellent cut-off between adjacent lines and ensure that no leak occurs between a closed and an open line, as is the case with tubing pinch valves. Such manifold stopcocks exist in various forms and are commercially available. The stopcocks 45-48 are driven by a set of motors 100-103. (Fig 5). The upper shafts 84-87 of these motors engage into the bottom portion of the respective stopcocks 45-48, using passing holes through the cabinet topdeck 88. As a safety measure to allow for possible manual actuation, the shafts can engage into the stopcock in one position only, a matching indent being provided for this purpose between the shafts and the stopcock. The motors can be stepper motors or DC motors with reducers. They are equipped with position encoders 104-107, whose signals are fed back to the microprocessor control unit, ensuring that the stopcocks are correctly positioned.

An alternative to using a manifold stopcock is a rotational multiport valve, as shown in Figs. 6 and 7. A

central rotor 127 is inserted into a stator 126. The rotor 127 can be frictionally rotated and can be engaged on the shaft of a motor. The central port 120, connected to the processing chamber 20, can be connected to the peripheral ports 121-125 by controlled rotation with angular steps of 72°. As a safety measure to allow for possible manual actuation, matching indents or other means can be provided in the rotor 127 and stator 126 for snap-holding the central port 120 in its selected angular positions aligned with the peripheral ports. A single motor is necessary to drive the rotor through the engagement recess 132. Two O-rings 130-131 ensure watertightness with the exterior (Fig.6).

The cabinet holding the instrumentation is shown in Fig. 8. It contains the cover 94 for holding the rotary seal 12 of the processing chamber 20. The cover 94 is made of two semi-circular disks that can rotate on hinge 89. An optical line sensor 83 allows the sensing of colors in the effluent tubing 51. It holds two LED-Photosensor channels, of different wavelength like red and green, and is capable of detecting the first cells coming out of the chamber 20. It can equally detect the empty state of the effluent line tubing when liquid is introduced in the chamber. The pressure port measurement 86 receives the bacterial filter 49 located on the disposable set. This allows the monitoring of the pressure in the processing chamber 20. Upper shafts 84-87 of the stopcock driver motors 100-103 (Fig. 5) are located behind the line sensor 83. An inclined module 90 receives the display 82 for user information and a keyboard 81 for controlling the instrumentation. A window 91 is located on the front panel 92 giving visibility to the chamber piston movement.

Application for Umbilical Blood Separation

Figs. 9.1 to 9.6 illustrate an application for umbilical cord blood separation. Bag 40 contains the umbilical cord blood rich in stem cells, recovered from the umbilical cord at birth of a child.

This bag 40 contains anticoagulant like CPD to avoid blood clots. Tubing line 53 is sterily connected to line 52 using a sterile connecting device or aseptically connected under laminar flow. However it is also possible to have the bag 40 preconnected to the whole set. The separation steps are:

Step 1 (Fig 9.1): Stopcocks 45 and 46 are rotated to connect bag 40 to the processing chamber 20. Centrifugation starts and initially is stabilized at a speed of 4000 rpm. The pneumatic system of the instrumentation establishes a vacuum to move the piston 21 downwards. Its speed is monitored by the optical sensor assembly 61 and 62, and the vacuum level is adjusted accordingly. If the volume of the bag 40 is smaller than the processing volume of chamber 20, effluent tubing line 51 will empty, which is detected by the optical line sensor 83. The piston 21 is maintained motionless by establishing a counter pressure through the pneumatic system, and the volume intaken into chamber 20 is recorded. The centrifuge speed is gradually increased to reach 6000 rpm, with pressure increased accordingly to keep piston 21 at the same position. In the case where the volume of bag 40 is larger than the processing volume of chamber 20, piston 21 will reach the bottom of the chamber, and the pneumatic system is turned off. In both cases, after a sedimentation time of about 5-8 min, centrifuge speed is slowly decreased, while maintaining a constant counter pressure below piston 21. Stopcocks 46,

47, 48 are rotated to establish a path between the processing chamber 20 and the plasma bag 43.

Step 2 (Fig. 9.2): When the centrifuge drops to a speed generally around 4000 rpm, the piston 21 starts to move upwards, with a preset speed allowing extraction rates around 100 ml/min. This value can be modified through the program parameters. Plasma starts to be collected in bag 43. When the volume of plasma extracted reaches approximately 40% of the volume intaken, the extraction rate will be reduced by half. The first platelets contained in the buffy-coat layer start to be extracted, which is detected by light absorbance in the optical line sensor 83. At a certain level of absorbance, which can be parametered by the control program, stopcock 47 is rotated to establish the path between the processing chamber 20 and bag 42.

Step 3 (Fig 9.3): A volume counter is triggered, and collection of a product very rich in stem cells starts. Extraction speed is always under the control of the piston optical sensor assembly 61 and 62. The volume counter value can be altered by the user in the program menu. It is chosen in order to encompass all the stem cell population, which has similar characteristics in density and size to the lymphocyte population. Such value corresponds generally to 20-30% of the intaken volume into the chamber. When this value is reached, stopcocks 47 and 48 are rotated to establish a path with bag 44.

Step 4 (Fig. 9.4): Centrifugation is generally stopped at this stage, and pressure diminished to allow a smooth extraction of the remaining red cells into bag 44. This phase is completed when piston 21 reaches the top of the processing chamber 20, detected by the piston optical sensor assembly 61 and 62. At this stage, if the bag 40 is not empty, the process will be resumed at Step 1,

otherwise it will proceed with Step 5, which is the transfer mode. An optional phase is to further separate the stem cell product by returning the content of bag 42 into the processing chamber 20, centrifuging the product once again and expressing its separated components to bags 43, 42 and 44 as before.

Step 5 (Fig. 9.5): Stopcocks 45 and 46 are rotated in order to establish a path between the preservative solution bag 41 and the processing chamber 20. The preservative solution is generally a composition based on 10 or 20vol% DMSO chemical solution, which can contain also a phosphate buffer. The centrifuge being idle, piston 21 is moved downwards by establishing a vacuum with the pneumatic system. The volume intaken is a proportion of the volume counter described in Step 4. When this proportion is reached the vacuum stops and stopcocks 46 and 47 are rotated in order to establish a path between the processing chamber 20 and the stem cell bag 42.

Step 6 (Fig. 9.6): The pneumatic system is turned down, and piston 21 moved upwards. The preservative solution is added to the content of the stem cell bag 42. This transfer phase is completed when the piston reaches the top of the processing chamber 20, detected by the piston optical sensor assembly 61 and 62. An optional additional phase can be added if the stem cell product needs to be diluted further with plasma. In this case Steps 5 and 6 will be repeated, with the difference that the transfer will be established between the plasma bag 43, the processing chamber 20 and the stem cell bag 42.

When all the steps described above are completed, all the stopcocks can be rotated at a 45° angle in order to close all the communicating ports. The bags 42-44 can be disconnected from the rest of the set, which can be discarded at this stage. The stem cell bag 42 is then

ready for conveying to a separate storage unit, the by-products plasma in bag 43 and red cells 44 being used for HLA typing and quality control assessments.

5 It will be appreciated that including a transfer mode, i.e. steps 5 and 6, opens up new applications for the system that were not available when the apparatus operated solely in the separation mode, in particular for applications requiring adding an additive solution to the separated components.

10 It is to be understood that this invention may be embodied in several different forms without departing from its spirit of essential characteristics. The scope of the invention is defined in the appended claims, rather than in the specific description preceding them. All
15 embodiments that fall within the meaning and range of equivalency of the claims are therefore intended to be embraced by the claims.

Moreover, the novel optical control device (60-71) herein described, as well as the arrangement of stopcocks
20 (45-48) and tubing, the multiport valve (Fig. 6 and 7), the special rotatable seal (1-7) for positive and negative pressure operation, and the special axial bearings mounting the chamber 20, permitting drive without a holding chuck, can all advantageously be used in different
25 systems.

CLAIMS

1. A system for the processing and separation of biological fluids into components, comprising a set of containers (40,42-44) for receiving the biological fluid to be separated and the separated components, and optionally one or more additional containers (41) for additive solutions, and a hollow centrifugal processing chamber (20) rotatable about an axis of rotation and having an axial inlet/outlet (7) for the biological fluid to be processed and for the processed components of the fluid, the processing chamber containing an axially movable member (21) which defines a separation space of variable size for receiving biological fluid, the member (21) being axially movable to intake a selected quantity of biological fluid to be processed into the separation space via said inlet and to express processed biological fluid components from the separation space via said outlet, and means (60-69) for monitoring the position of the axially movable member (21) to thereby control the amount of intaken biological fluid and the expression of separated components, the system further comprising a distribution valve arrangement (45-48;120-127) for establishing selective communication between the processing chamber (20) and selected containers (40-44) or for placing the processing chamber and containers out of communication, the system being arranged to operate in a separation mode and in a non-separation transfer mode, wherein:

- in the separation mode fluids can be intaken into the processing chamber (20) while the chamber is rotating or stationary, fluid intaken into the chamber is centrifuged and separated into components, and the separated components expressed while the chamber is

rotating or, optionally, for the last separated component, while the chamber is stationary; and

- in the transfer mode the processing chamber (20) intakes fluid and expresses fluid with the chamber stationary, the valve actuation arrangement (45-48;120-127) being actuatable to transfer amounts of fluid from one container (40-44) to another via the processing chamber (20), by axially moving the member (21), without centrifugation or separation of the fluid into components, and said means (60-69) for monitoring the position of the axially movable member (21) controls the amounts of non-separated fluids transferred.

2. The system of claim 1, wherein the distribution valve arrangement comprises a plurality of stopcock valves (45-48) connected to tubing lines interconnecting the set of containers (40,42-44), the optional additional containers (41), the processing chamber (20) and further stopcock valves, each stopcock valve (45-48) comprising a rotatable stopcock valve member having a shaft (84-87) associated with drive means (100-103), the rotation of said shaft selectively connecting or disconnecting the stopcock valve's tubing lines.

3. The system of claim 2, comprising means for allowing insertion of each stopcock valve (45-48) only in a defined angular alignment of the rotatable stopcock valve member.

4. The system of claim 1, wherein the distribution valve arrangement comprises a multiport valve comprising a central rotor (127) rotatably mounted in an annular stator (126), the rotor having a central port (120) connected to the processing chamber (20) and leading to the rotor outer periphery, and the stator having a plurality of ports (121-125) at selected angular locations each connected to a container (40-44) and each leading into the inner

periphery of the annular stator, the central port (120) of the rotor being connectable to selected ports (121-125) of the stator (126), or disconnected, by rotation of the rotor (127).

5 5. The system of any preceding claim, wherein the movable member is a piston (21) fluid-tightly movably mounted in a generally-cylindrical centrifugal processing chamber (20).

10 6. The system of claim 5, further comprising optical means for monitoring the position of the piston (21), comprising an alignment of light emitting elements (60) generally parallel to the piston axis, and an alignment of light receiving elements (61) generally parallel to the piston axis, the receiving elements (61)
15 being arranged to receive light from the emitting elements (60) transmitted through or past the piston (21) or reflected by the piston (21), and delivering a signal (62) representative of the piston's position.

20 7. The system of claim 6, wherein said signal (62) is delivered to means (70,71) for moving the piston (21) and means (60-65) for controlling the piston's position.

25 8. The system of any preceding claim, wherein the axial inlet/outlet comprises a rotatable seal (3-6) mountable in a stationary housing (1-2), said seal being operable for positive and negative pressure conditions in the rotatable chamber (20).

30 9. The system of any preceding claim, wherein the processing chamber (20) is mounted for rotation about its axis by means of bearings (9; 72, 73) at opposite ends of the chamber, one end of the chamber (20) being associated with means (55-57) for rotating the chamber (20) without any support at the chamber periphery.

10. A method of processing and separating biological fluids in a system according to any preceding claim, the method comprising:

5 separating a biological fluid with the system operating in the separation mode, by intaking fluid into the processing chamber (20) while the chamber is rotating or stationary, centrifuging fluid intaken into the chamber (20) to separate the fluid into components, and expressing the separated components while the chamber is rotating or
10 possibly, for the last component, while the chamber is stationary; and

transferring fluid between containers (40-44) with the system operating in the transfer mode, by intaking fluid into the processing chamber (20) with the chamber
15 stationary, actuating the valve distribution arrangement (45-48;120-127) to transfer an amount of fluid from one container (40-44) to another via the processing chamber (20), by moving the member (21), without centrifugation or separation of the fluid into components, and monitoring
20 the position of the movable member (21) to control the amount of non-separated fluid transferred.

11. The method of claim 10, wherein a component of the biological fluid is separated into a given container (42), the amount of said component separated into the
25 given container (42) being controlled by monitoring the position of said member (21), and an additive solution is transferred from an additional container (41) to said given container (42) via the processing chamber (20) in said transfer mode, the amount of additive solution
30 transferred being calculated as a function of the amount of said separated component in the given container (42).

12. The method of claim 9 or 10, wherein a density gradient product and blood are introduced into the processing chamber (20), and a component of the biological

fluid is separated into a given container (42) and its collection is completed when the density gradient appears.

5 13. A disposable set for collecting and separating selected quantities of biological fluids comprising the centrifugal processing chamber (20) of a system according to any one of claims 1 to 8, wherein the inlet/outlet of the centrifugal processing chamber (20) is connected to a container (40) of biological fluid, an additional container (41) containing an additive solution, a
10 plurality of containers (42-44) for receiving the separated components of the biological fluid, interconnected by a plurality of stopcock valves as defined in claim 2 or by a multiport valve as defined in claim 3.

15 14. Use of the system of any one of claims 1 to 9, for processing variable volumes of biological fluid from 10 ml up to the maximum volume of the separation chamber (20), and for adding an additive solution to the separated components.

20 15. Use according to claim 14 for separation of stem cells from blood and mixing the separated stem cells with a preservative solution.

25 16. Use according to claim 15, for separation of hematopoietic stem cells from umbilical cord blood, from an apheresis collection, or from a bone marrow aspirate.

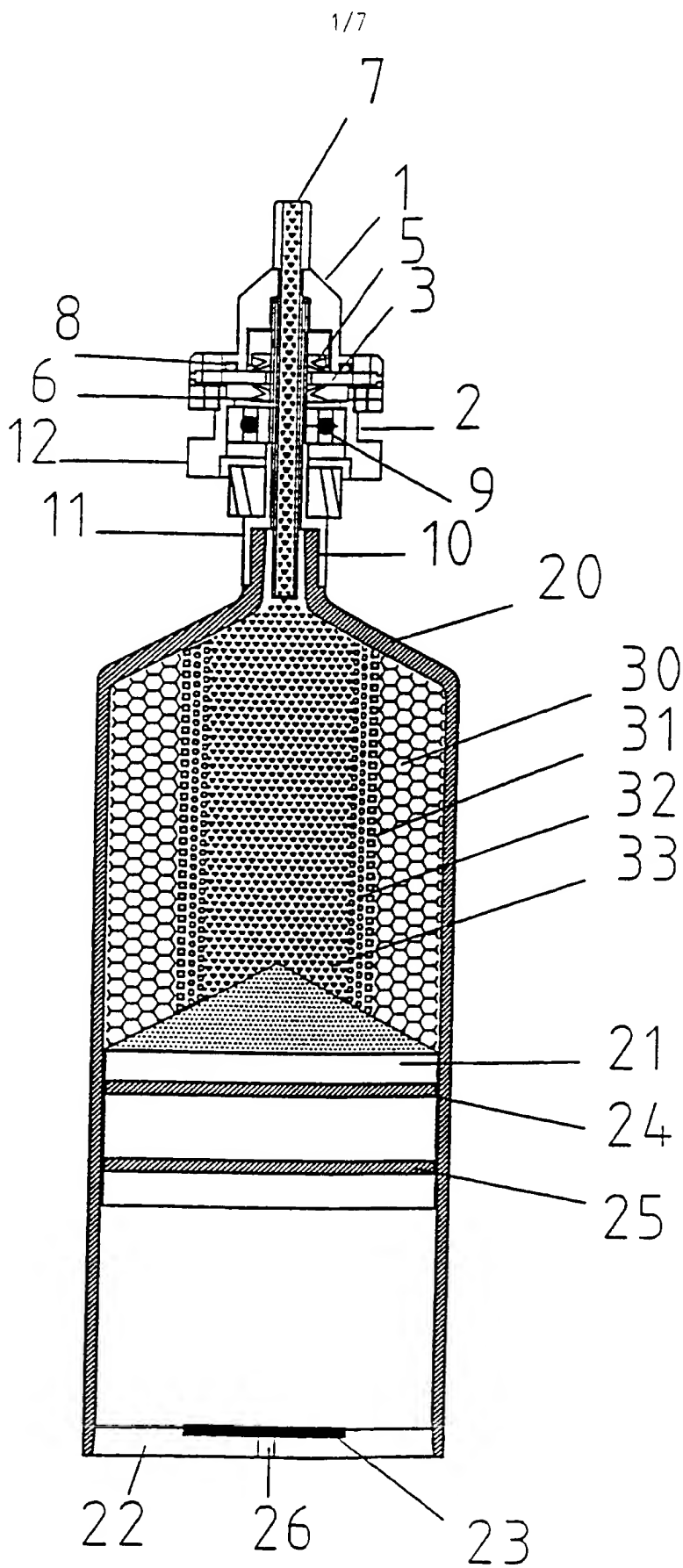


Fig. 1

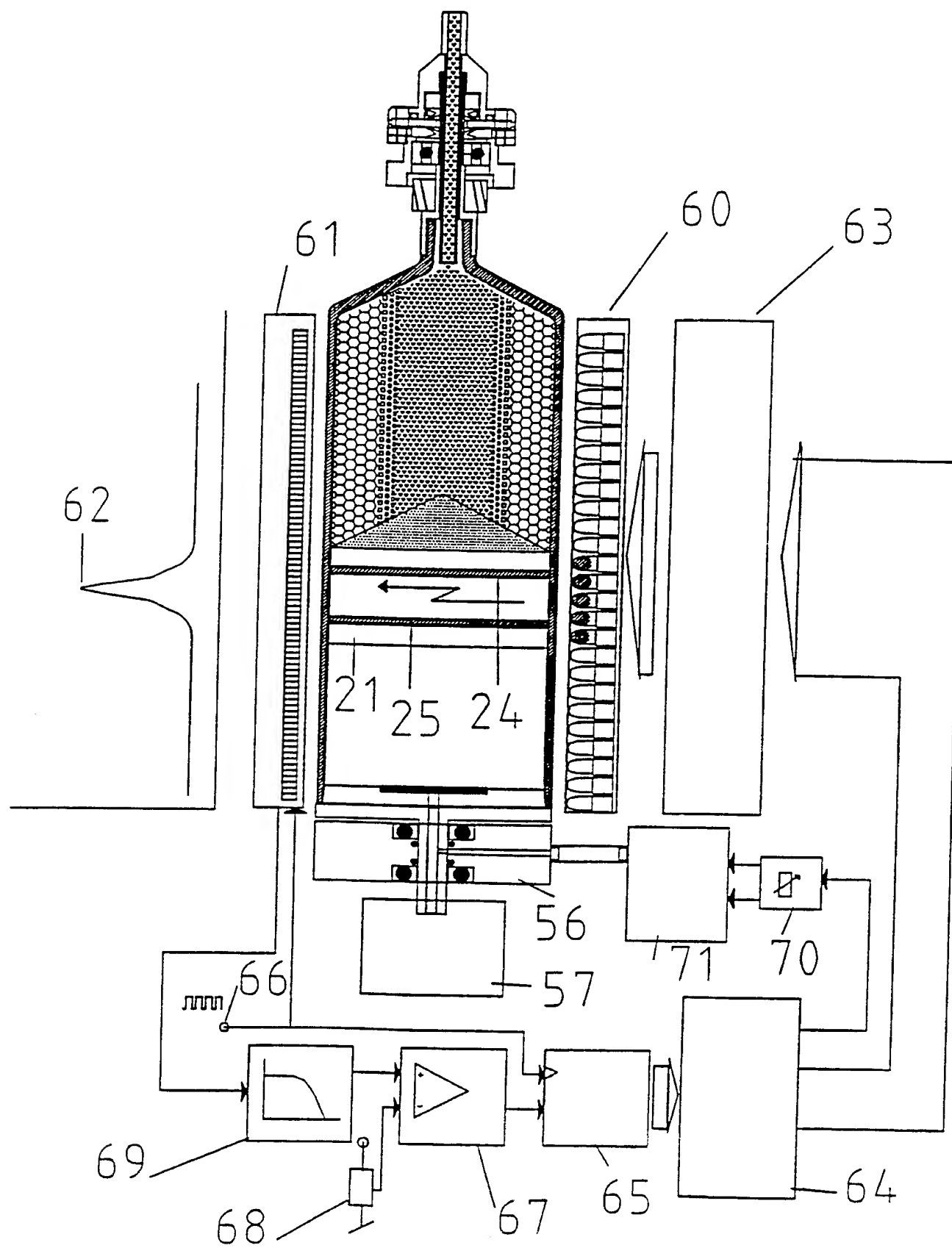


Fig. 2

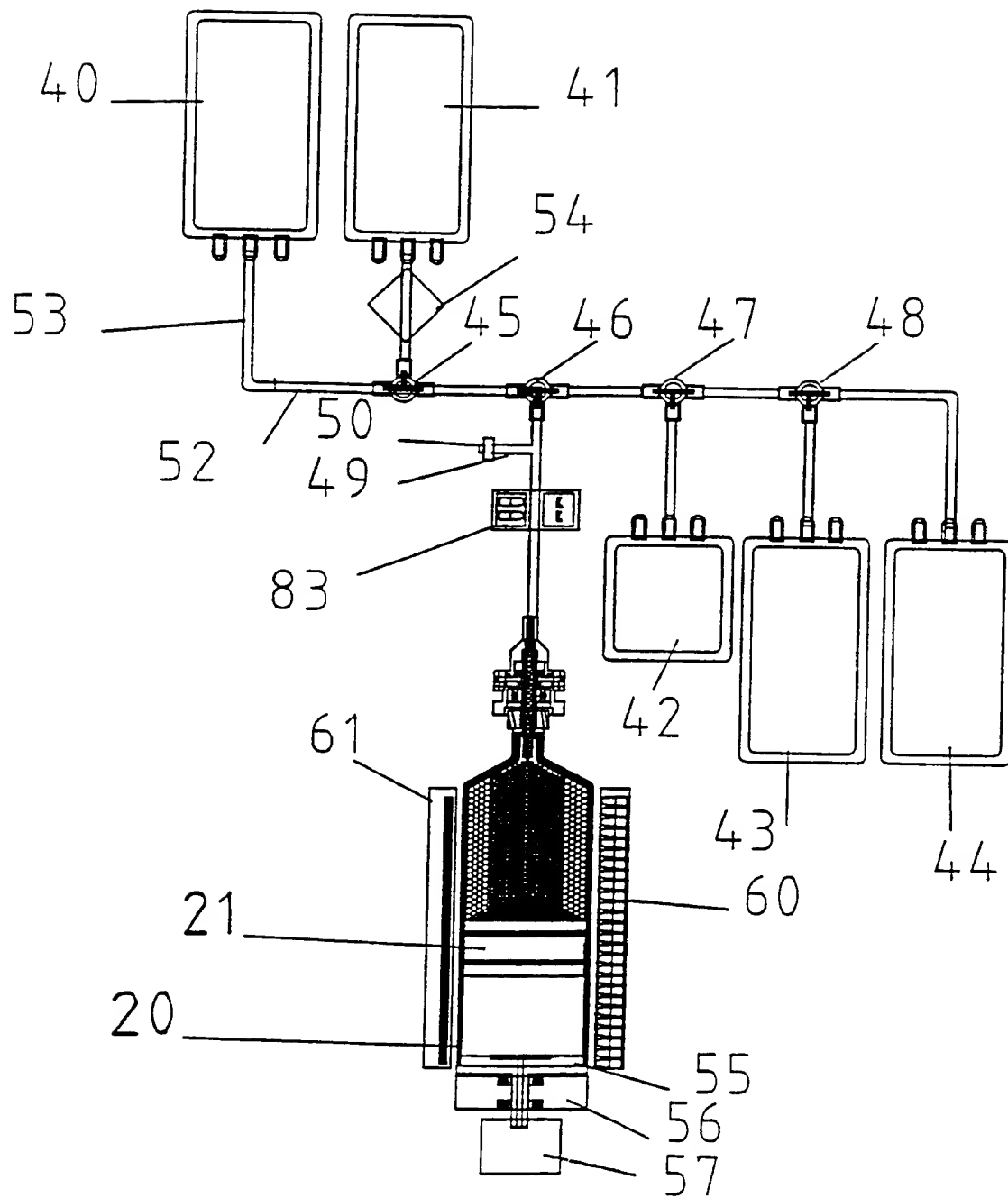


Fig. 3

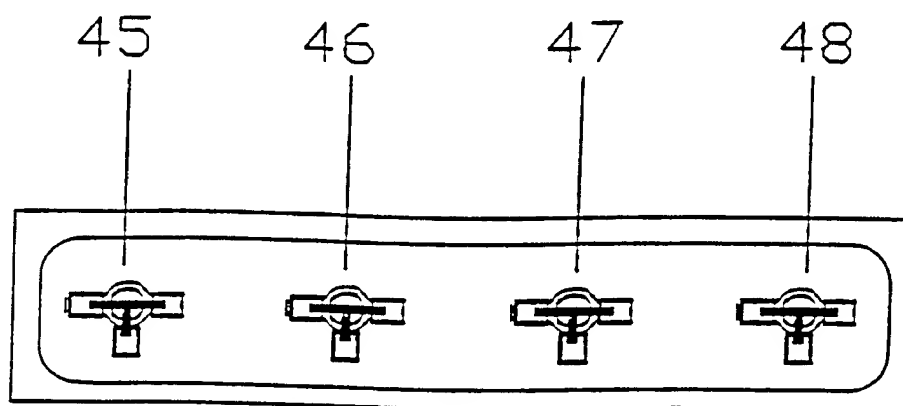


Fig. 4

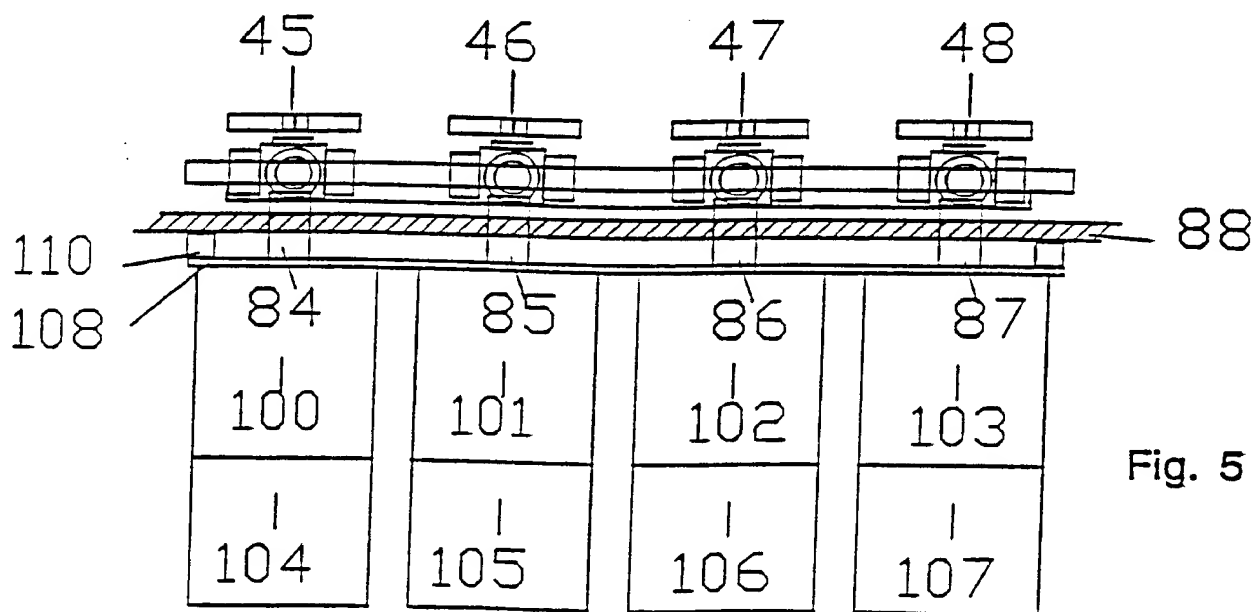
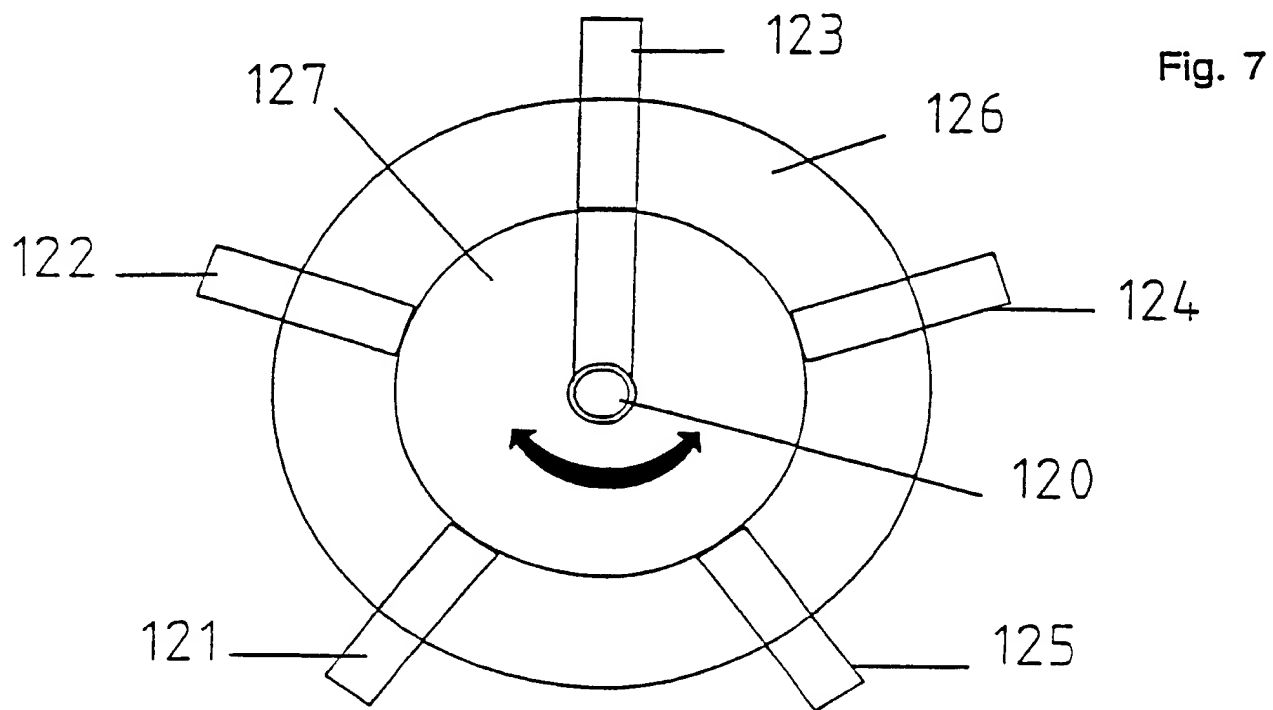
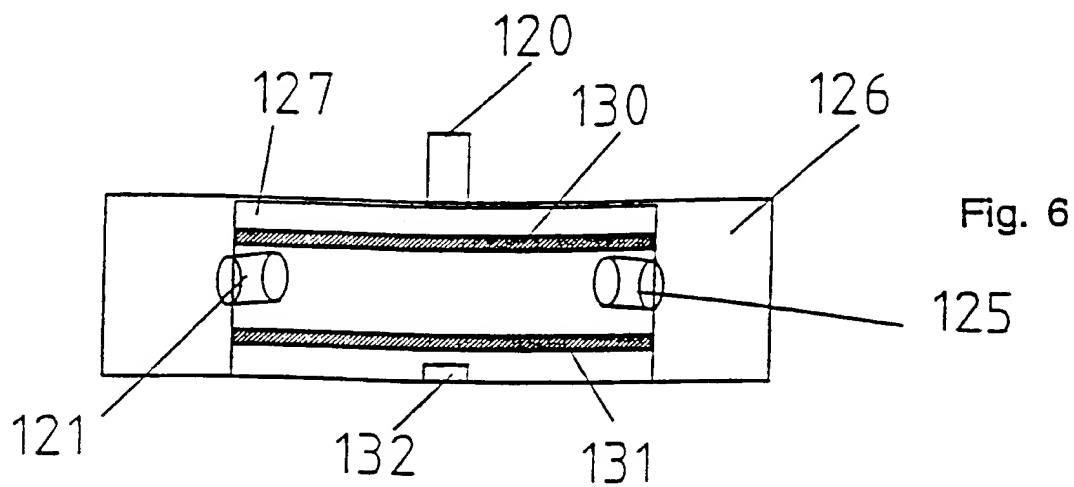


Fig. 5



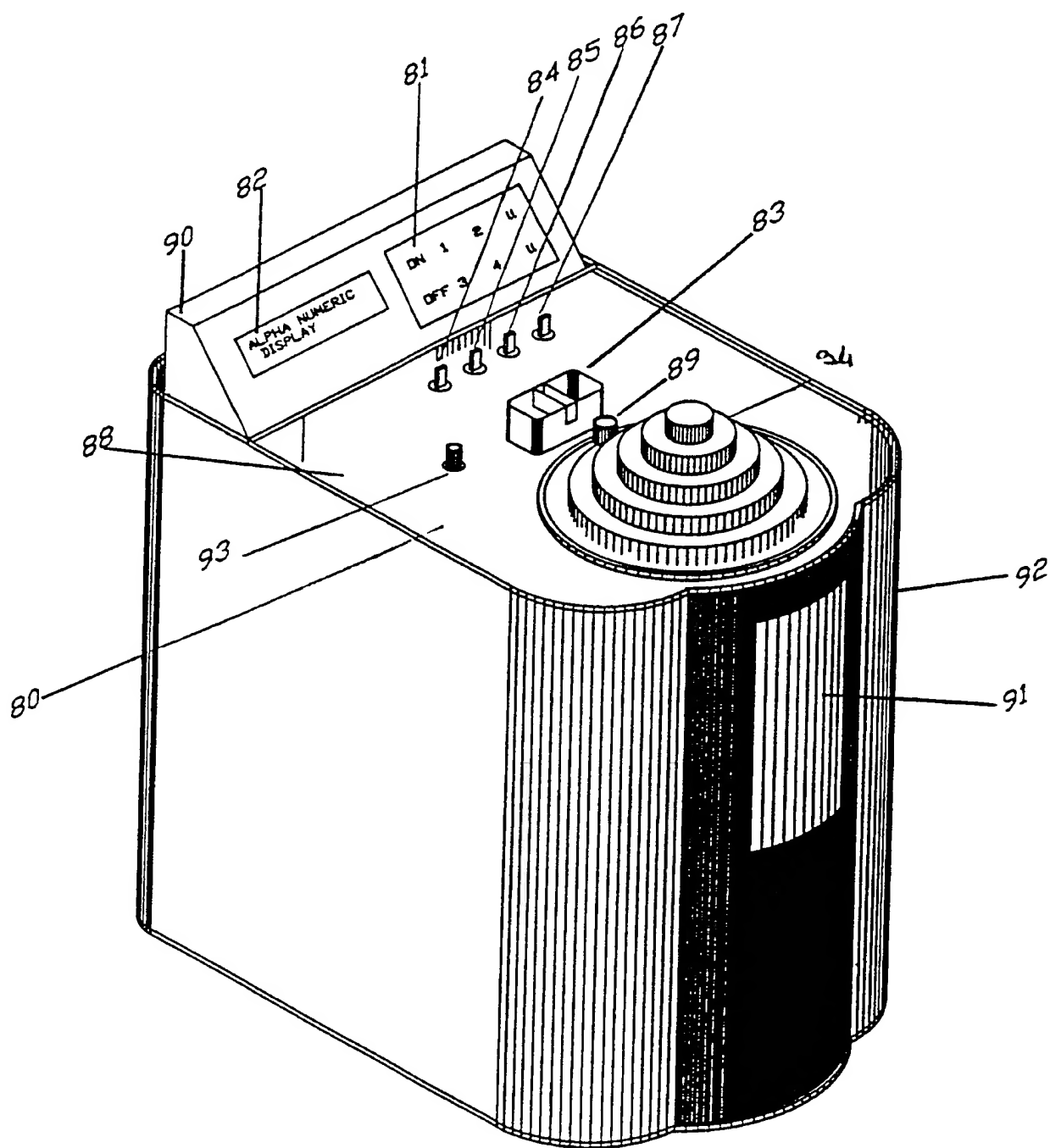


Fig. 8

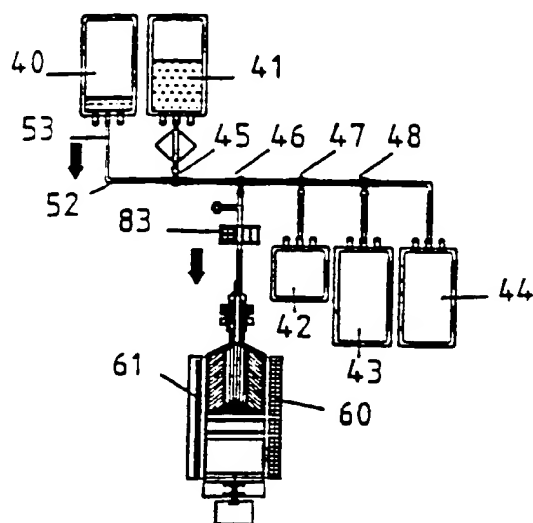


Fig. 9.1

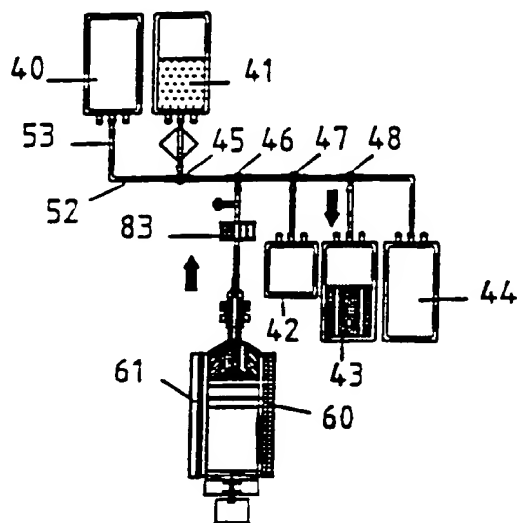


Fig. 9.2

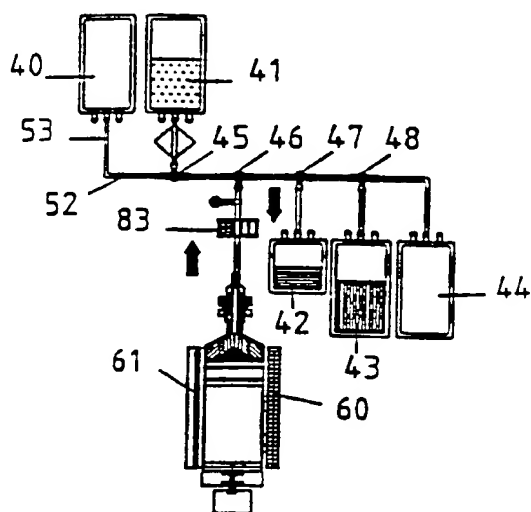


Fig. 9.3

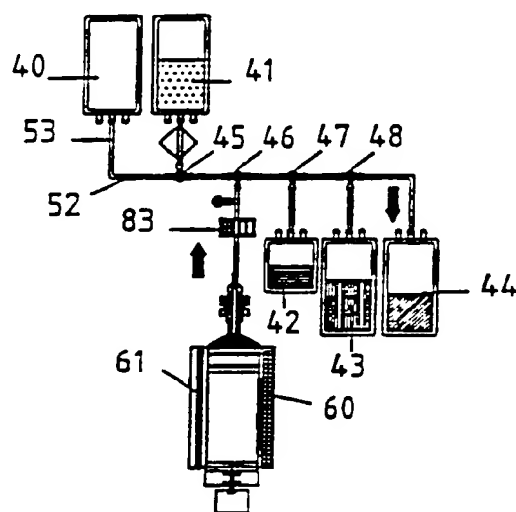


Fig. 9.4

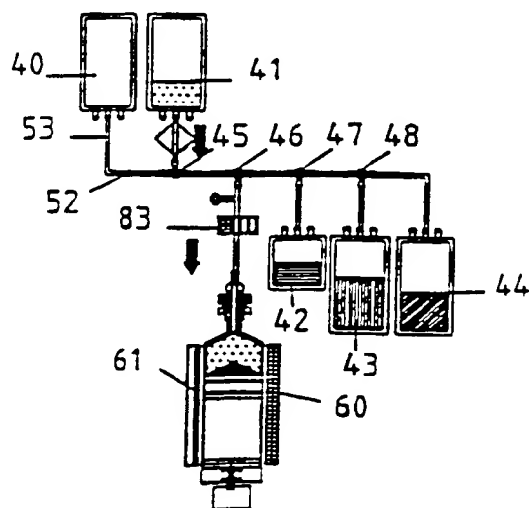


Fig. 9.5

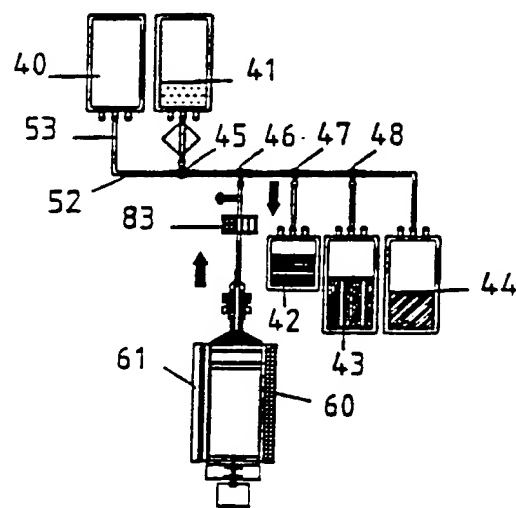


Fig. 9.6

for placing the processing chamber and containers out of communication.

The system according to EP-B-0 912 250 is designed to operate for the separation of biological fluids, and has proven to be very polyvalent for many separation applications, especially for on-line separation of components from a donor or a patient.

DISCLOSURE OF THE INVENTION

According to the invention, such system is arranged to operate in a separation mode as defined in claim 1 and in a non-separation transfer mode, which provides greater possibilities for use of the system including new applications which were heretofore not contemplated, such as separation of hematopoietic stem cells and in general laboratory processing. According to the invention, the system comprises means for controlling operation of the system in two operational modes, a separation mode and a non-separation transfer mode, wherein:

- in the separation mode fluids can be intaken into the processing chamber while the chamber is rotating or stationary, fluid intaken into the chamber is centrifuged and separated into components, and the separated components expressed while the chamber is rotating or, optionally, for the last separated component, while the chamber is stationary; and

- in the transfer mode the processing chamber intakes fluid and expresses fluid with the chamber stationary. The valve actuation arrangement is actuatable to transfer amounts of fluid from one container to another via the processing chamber, by moving the member, without centrifugation or separation of the fluid into components, and the means for monitoring the position of the movable member controls the amounts of non-separated fluids transferred.

reflection light from the piston 61 rather in transmittance light through the piston 21.

The disposable set (Fig 3) is composed of bags 40-44, tubing lines connected to stopcocks 45-48, and the processing chamber 20. Bag 40 contains the umbilical cord blood to process. Bag 41 contains preservative solution, generally based on a DMSO (Dimethyl Sulfoxide) solution. It is connected to the disposable set through a bacterial 0.2 micron filter 54. Bag 42 is the collection bag for the stem-cell rich product. Its plastic composition is made of a material suiting long term storage. Bag 43 is the collection bag for the plasma and bag 44 is the one for the red cells.

Fig. 3 also shows means for rotating the chamber 20 by contacting the chamber's bottom 22 with a rotary disc 55 without any support at the chamber periphery.

An array of stopcocks 45-48 (Fig. 5) organized in a manifold 58 allows the connection between the different tubing lines. These rotational stopcocks provide an excellent cut-off between adjacent lines and ensure that no leak occurs between a closed and an open line, as is the case with tubing pinch valves. Such manifold stopcocks exist in various forms and are commercially available. The stopcocks 45-48 are driven by a set of motors 100-103. (Fig 5). The upper shafts 84-87 of these motors engage into the bottom portion of the respective stopcocks 45-48, using passing holes through the cabinet topdeck 88. As a safety measure to allow for possible manual actuation, the shafts can engage into the stopcock in one position only, a matching indent being provided for this purpose between the shafts and the stopcock. The motors can be stepper motors or DC motors with reducers. They are equipped with position encoders 104-107, whose signals are fed back to the microprocessor control unit, ensuring that the stopcocks are correctly positioned.

An alternative to using a manifold stopcock is a rotational multiport valve, as shown in Figs. 6 and 7. A

CLAIMS

1. A system for the processing and separation of biological fluids into components, comprising a set of containers (40,42-44) for receiving the biological fluid to be separated and the separated components, and optionally one or more additional containers (41) for additive solutions, and a hollow centrifugal processing chamber (20) rotatable about an axis of rotation and having an axial inlet/outlet (7) for the biological fluid to be processed and for the processed components of the fluid, the processing chamber containing an axially movable member (21) which defines a separation space of variable size for receiving biological fluid, the member (21) being axially movable to intake a selected quantity of biological fluid to be processed into the separation space via said inlet and to express processed biological fluid components from the separation space via said outlet, and means (60-69) for monitoring the position of the axially movable member (21) to thereby control the amount of intaken biological fluid and the expression of separated components, the system further comprising a distribution valve arrangement (45-48;120-127) for establishing selective communication between the processing chamber (20) and selected containers (40-44) or for placing the processing chamber and containers out of communication, the system comprising means for controlling operation of the system in two operational modes, a separation mode and a non-separation transfer mode, wherein:

- in the separation mode fluids can be intaken into the processing chamber (20) while the chamber is rotating or stationary, fluid intaken into the chamber is centrifuged and separated into components, and the separated components expressed while the chamber is rotating or, optionally, for

the last separated component, while the chamber is stationary; and

- in the transfer mode the processing chamber (20) intakes fluid and expresses fluid with the chamber stationary, the valve actuation arrangement (45-48;120-127) being actuatable to transfer amounts of fluid from one container (40-44) to another via the processing chamber (20), by axially moving the member (21), without centrifugation or separation of the fluid into components, and said means (60-69) for monitoring the position of the axially movable member (21) controls the amounts of non-separated fluids transferred.

2. The system of claim 1, wherein the distribution valve arrangement comprises a set of rotational stopcock valves (45-48) arranged in a manifold array (58), or a multiport rotational valve (120-132).

3. The system of claim 1 or 2, wherein the distribution valve arrangement comprises a plurality of stopcock valves (45-48) connected to tubing lines interconnecting the set of containers (40,42-44), the optional additional containers (41), the processing chamber (20) and further stopcock valves, each stopcock valve (45-48) comprising a rotatable stopcock valve member having a shaft (84-87) associated with drive means (100-103), the rotation of said shaft selectively connecting or disconnecting the stopcock valve's tubing lines.

4. The system of claim 3, comprising means for allowing insertion of each stopcock valve (45-48) only in a defined angular alignment of the rotatable stopcock valve member.

5. The system of claim 1 or 2, wherein the distribution valve arrangement comprises a multiport valve comprising a central rotor (127) rotatably mounted in an annular stator (126), the rotor having a central port (120) connected to the processing chamber (20) and leading to the rotor outer periphery, and the stator having a plurality of ports (121-125) at selected angular locations each connected to a

container (40-44) and each leading into the inner periphery of the annular stator, the central port (120) of the rotor being connectable to selected ports (121-125) of the stator (126), or disconnected, by rotation of the rotor (127).

6. The system of any preceding claim, wherein the movable member is a piston (21) fluid-tightly movably mounted in a generally-cylindrical centrifugal processing chamber (20).

7. The system of claim 6, further comprising optical means for monitoring the position of the piston (21), comprising an alignment of light emitting elements (60) generally parallel to the piston axis, and an alignment of light receiving elements (61) generally parallel to the piston axis, the receiving elements (61) being arranged to receive light from the emitting elements (60) transmitted through or past the piston (21) or reflected by the piston (21), and to deliver a signal (62) representative of the piston's position.

8. The system of claim 7, wherein the receiving elements (61) are arranged to deliver said signal (62) to means (70,71) for moving the piston (21) and means (60-65) for controlling the piston's position.

9. The system of any preceding claim, comprising an optical sensor (83) monitoring fluid in the tubing line (51) connected to the axial inlet/outlet (7), for stopping the intake of biological fluid when the tubing line (51) is empty during the intake mode and/or for providing a signal for switching the distribution valve arrangement (45-48;120-127) in the extraction mode.

10. The system of any preceding claim, wherein the axial inlet/outlet comprises a rotatable seal (3-6) mountable in a stationary housing (1-2), said seal being operable for

positive and negative pressure conditions in the rotatable chamber (20).

11. The system of any preceding claim, wherein the processing chamber (20) is mounted for rotation about its axis by means of bearings (9; 72, 73) at opposite ends of the chamber, one end of the chamber (20) being associated with means (55-57) for rotating the chamber (20) by contacting the chamber's bottom (22) with a rotary disc (55) without any support at the chamber periphery.

12. The system of any preceding claim, wherein the means for controlling operation of the system in said two operational modes comprises a microprocessor based control system controlling an automated protocol.

13. A method of processing and separating biological fluids in a system according to any preceding claim, the method comprising:

separating a biological fluid with the system operating in the separation mode, by intaking fluid into the processing chamber (20) while the chamber is rotating or stationary, centrifuging fluid intaken into the chamber (20) to separate the fluid into components, and expressing the separated components while the chamber is rotating or possibly, for the last component, while the chamber is stationary; and

transferring fluid between containers (40-44) with the system operating in the transfer mode, by intaking fluid into the processing chamber (20) with the chamber stationary, actuating the valve distribution arrangement (45-48;120-127) to transfer an amount of fluid from one container (40-44) to another via the processing chamber (20), by moving the member (21), without centrifugation or separation of the fluid into components, and monitoring the position of the movable member (21) to control the amount of non-separated fluid transferred.

14. The method of claim 13, wherein a component of the biological fluid is separated into a given container (42), the amount of said component separated into the given container (42) being controlled by monitoring the position of said member (21), and an additive solution is transferred from an additional container (41) to said given container (42) via the processing chamber (20) in said transfer mode, the amount of additive solution transferred being calculated as a function of the amount of said separated component in the given container (42).

15. The method of claim 13 or 14, wherein a density gradient product and blood are introduced into the processing chamber (20), and a component of the biological fluid is separated into a given container (42) and its collection is completed when the density gradient appears.

16. The method of any one of claims 13 to 15, wherein operation of the system in said two operational modes is controlled according to an automated protocol by a microprocessor based control system.

17. A disposable set for collecting and separating selected quantities of biological fluids comprising the centrifugal processing chamber (20) of a system according to any one of claims 1 to 12, wherein the inlet/outlet of the centrifugal processing chamber (20) is connected to a container (40) of biological fluid, an additional container (41) containing an additive solution, a plurality of containers (42-44) for receiving the separated components of the biological fluid, interconnected by a distribution valve arrangement comprising a set of rotational stopcock valves (45-48) arranged in a manifold array (58), or a multiport rotational valve (120-132).

18. The disposable set of claim 17, wherein the distribution valve arrangement comprises a plurality of stopcock valves as defined in claim 3.

19. The disposable set of claim 17, wherein the distribution valve arrangement comprises a multiport valve as defined in claim 3.

20. Use of the system of any one of claims 1 to 12, for processing variable volumes of biological fluid from 10 ml up to the maximum volume of the separation chamber (20), and for adding an additive solution to the separated components.

21. Use according to claim 20, for separation of stem cells from blood and mixing the separated stem cells with a preservative solution.

22. Use according to claim 21, for separation of hematopoietic stem cells from umbilical cord blood, from an apheresis collection, or from a bone marrow aspirate.

informal

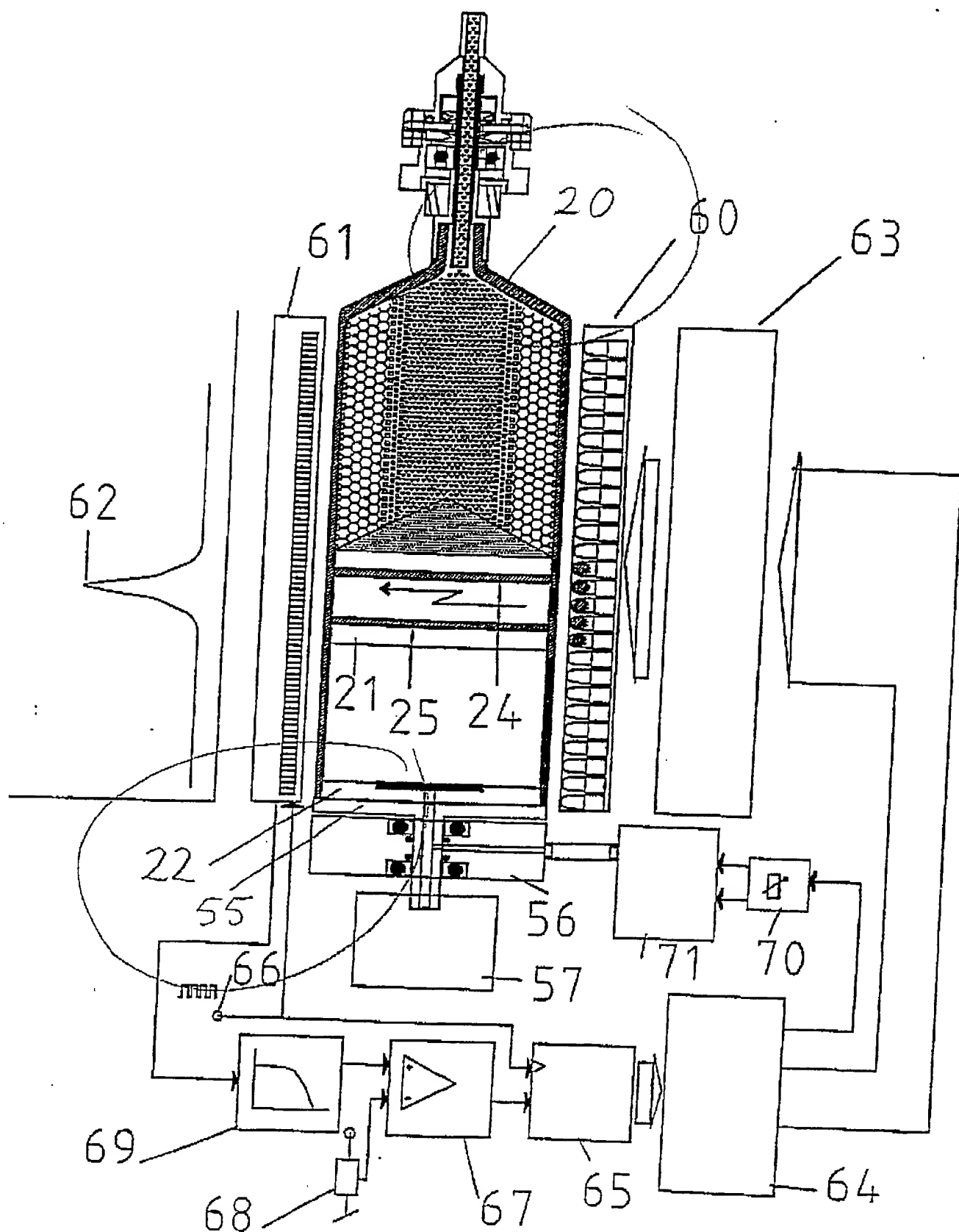


Fig. 2

8/01/01

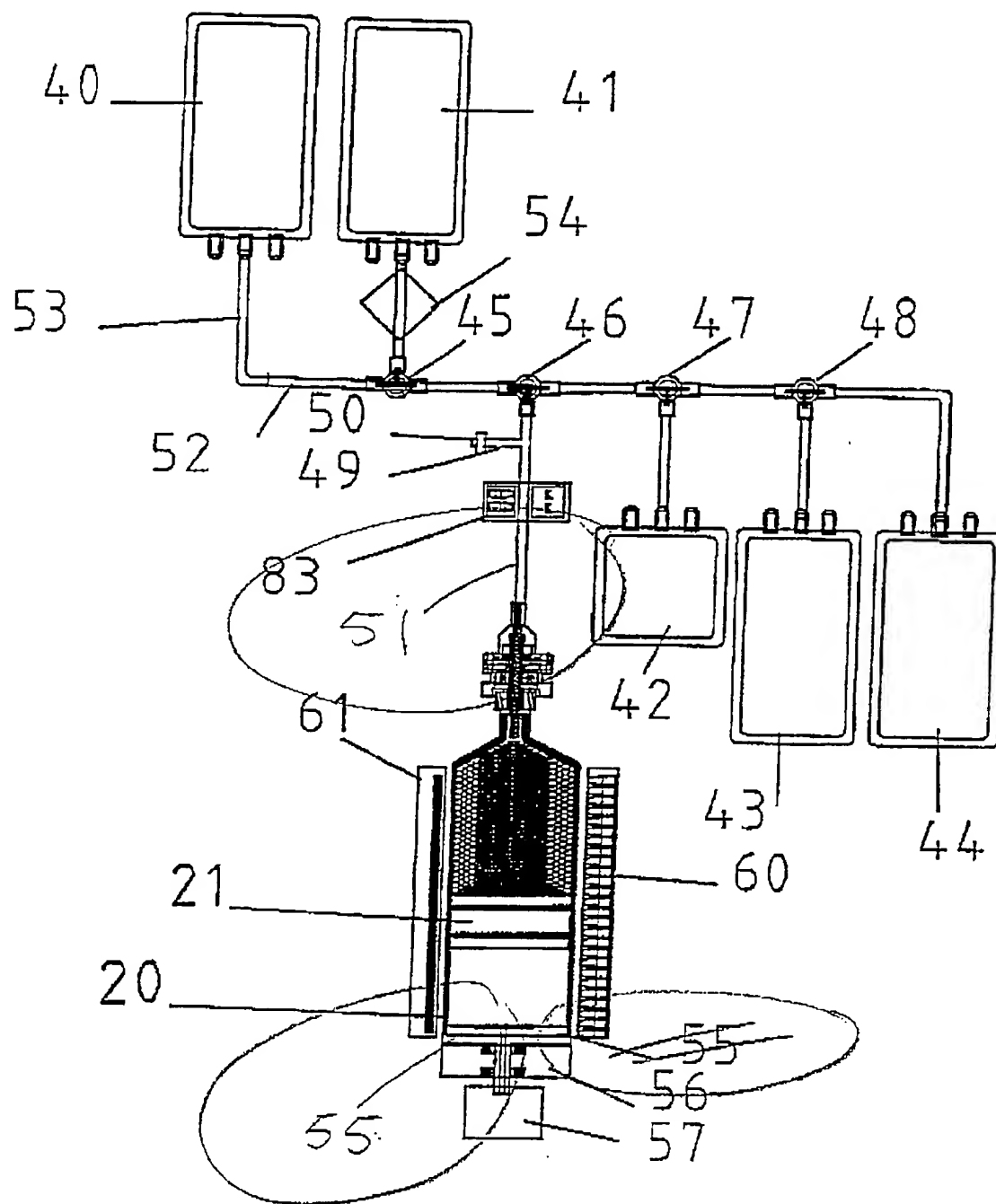
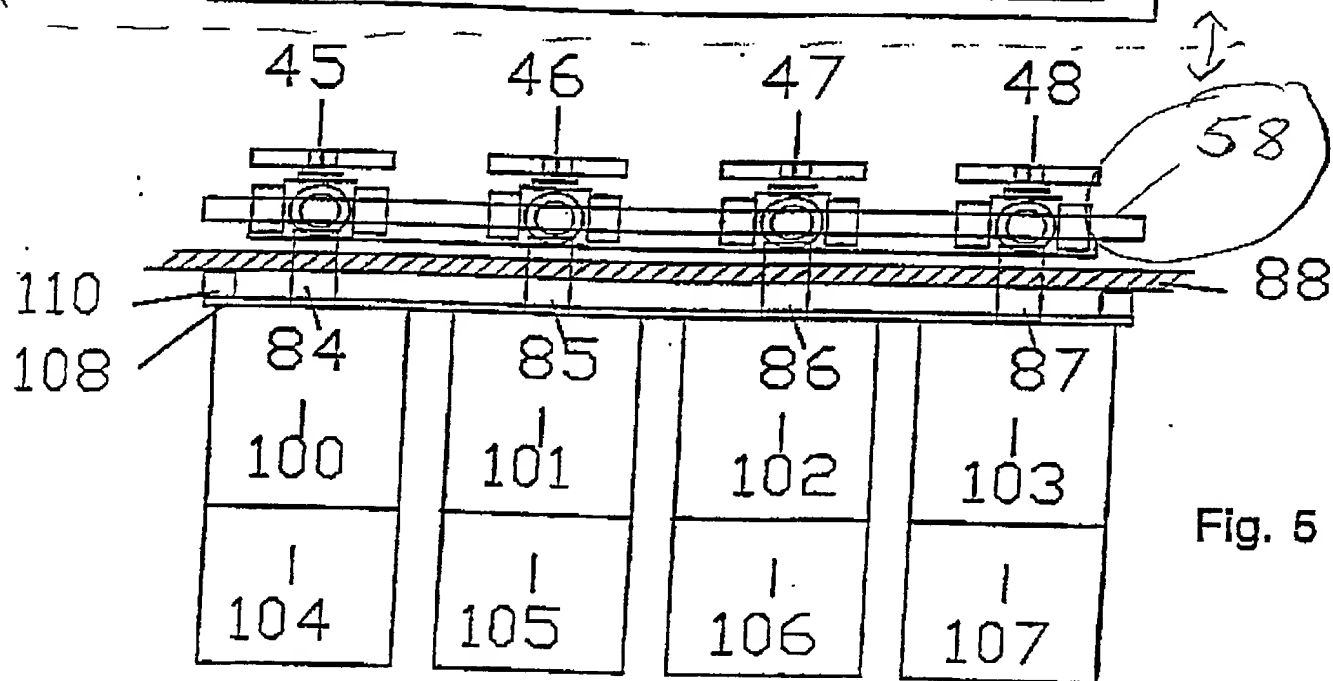
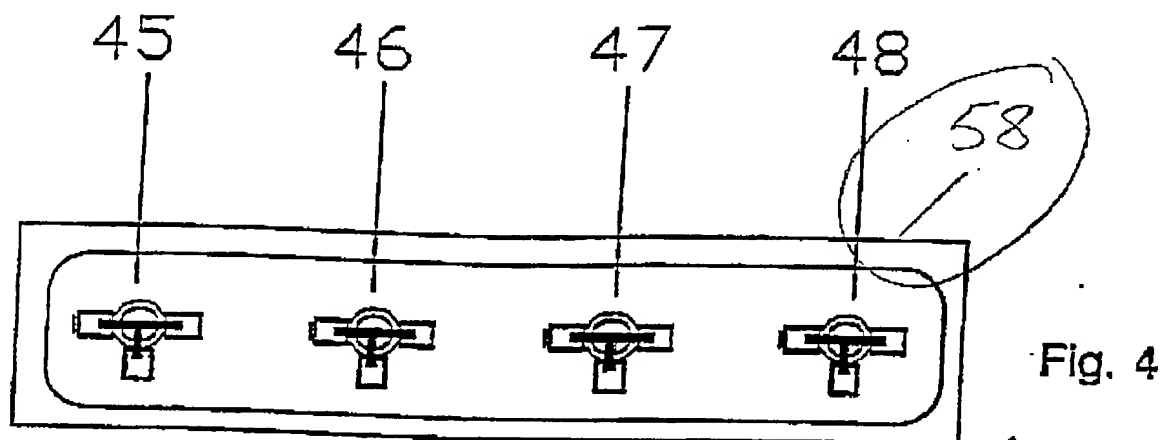
informal copy

Fig. 3

8/01/01

informal copy

8/01/01

